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Use of Tricaine Methanesulfonate (MS222) for Euthanasia of Reptiles

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Tricaine methanesulfonate (MS222) injected into the intracoelomic cavity of reptiles was evaluated as a chemical euthanasia method. Three western fence lizards, 2 desert iguanas, 4 garter snakes, and 6 geckos were euthanized by intracoelomic injection of 250 to 500 mg/kg of 0.7% to 1% sodium-bicarbonate–buffered MS222 solution followed by intracoelomic injection of 0.1 to 1.0 ml unbuffered 50% (v/v) MS222 solution. A simple 2-stage protocol for euthanasia of reptiles by using MS222 is outlined. In addition, the conditions for safe use of MS222 are discussed. MS222 offers an alternative to sodium pentobarbital for euthanasia of reptiles.

Abbreviations: AVMA, American Veterinary Medical Association; MS222, tricaine methanesulfonate.

Euthanasia of reptiles is often required for research as well as clinical purposes. The Public Health Service Policy on Humane Care and Use of Laboratory Animals[21] requires that euthanasia of ectotherms be consistent with the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.[1] Euthanasia techniques, according to these guidelines, "should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function." However, this objective may be difficult to achieve in many reptiles. Intravenous injection of sodium pentobarbital, the only chemical agent listed in the AVMA Guidelines as acceptable for euthanasia of reptiles, is considered the euthanasia method of choice.[15,16] However, even in the hands of trained personnel, this method can be difficult or impossible to use in turtles, aggressive animals, or any animal in which vascular access is difficult. Sodium pentobarbital can be injected into the coelomic cavity of reptiles, but many authors, including those contributing to the AVMA Guidelines, concede that death can take 30 min or longer when pentobarbital is administered this way.[1,3,15,16] Some authors do not advocate the use of pentobarbital for euthanasia of reptiles by any route,[8] because of this lengthy time to death. Further, concentrated pentobarbital solutions that are formulated for intravenous use are highly alkaline and may cause irritation of tissues and pain when injected extravascularly.[3,23]

Depending on its formulation, sodium pentobarbital is classified as a Class II or Class III agent by the US Drug Enforcement Agency. This assignment makes it potentially difficult to procure pentobarbital and authorize its use. Many institutions require background security checks before personnel can buy or use sodium pentobarbital. Complicated secure storage and documentation practices are required when working with pentobarbital, making its field use particularly cumbersome. Further, in our experience, this chemical has caused postmortem 'kinking' in reptiles, particularly snakes, near the point of injection, leading to asymmetric or nonstandard museum specimens. This effect hinders the collection of basic data, such as measuring body length in morphologic studies.

Few recognized alternative methods to sodium pentobarbital for chemical euthanasia for reptiles are available[1,3,10,16,27] although several different methods for euthanasia have been noted.[5,22] Unfortunately, descriptions of alternative methods to pentobarbital lack detailed information about how chemicals were prepared, the dose required for satisfactory euthanasia, and observations on behavioral reactions (that is, pain response). Furthermore, these methods are not approved by the AVMA, due in part to lack of research evaluating these methods.

Physical methods, such as pithing or decapitation followed by pithing, are appropriate physical methods of euthanasia in some reptiles and are conditionally acceptable according to the AVMA guidelines. However, these methods can be difficult to perform, particularly in turtles, and therefore inhumane. Further, natural history museums often collect specimens that will be preserved as vouchers to be used for a variety of purposes.[20] Morphologic studies or verification of identification in cryptic species, for example, requires an intact, prepared carcass. Methods of euthanasia that are disfiguring, such as decapitation, are clearly inappropriate for this purpose. The lack of an ideal euthanasia method in reptiles has been recognized, and a 1989 monograph[11] that is still considered the 'gold standard' for euthanasia of reptiles and amphibians[16] states that "an urgent need exists for research into the euthanasia of lower vertebrates."

The chemical tricaine methanesulfonate (MS222) is an acceptable agent for euthanasia in fish and aquatic amphibians.[1,2,7,9,10,16,18,27] The standard protocol for euthanasia of these species is immersion in a pH-neutralized solution for 15 to 30 min, followed by a secondary physical euthanasia method. Intracoelomic administration of concentrated MS222 is considered acceptable for euthanasia of fish and amphibians,[1,2,7,9,27] although the concentration(s) applicable are unclear. Both the Canadian[9] and AVMA euthanasia guidelines[1], under the general heading of fish, amphibians, and reptiles, state that MS222 may be given by 'various routes,' although the references cited in the guidelines address fish only. Cardiac arrest was produced in leopard frogs (*Rana pipiens*) injected intracoelomically with 250 mg/kg MS222.[25] MS222 administered by the intracoelomic and intramuscular routes has been evaluated or cited as an anesthetic drug for terrestrial frogs and reptiles.[12,14,24,25]

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In contrast, the European recommendations[10] state that the use of MS222 for reptile euthanasia is unacceptable, although the guidelines cite no studies to defend this position. The publication[10] anecdotally notes that intramuscular injection of MS222 has been used in snakes and alligators and states that due to the lack of information on its humaneness, using MS222 for euthanasia of reptiles is not considered acceptable. The Canadian guidelines[8] also list MS222 (as well as other injectable anesthetics, including pentobarbital) as unacceptable for euthanasia of reptiles. This decision is attributed to the difficulty of intravenous injection or the lengthy time to death when injecting by other routes.

MS222 dissolved in tap or distilled water is acidic. In addition to the animal welfare concerns of exposing potentially sensitive tissues by injecting acidic solutions into live vertebrates, experimentation has shown that MS222 is absorbed more readily across lipid membranes at more neutral conditions and is therefore more efficacious.[19] The concept of using concentrated MS222 for injection is further complicated because buffering of highly concentrated MS222 causes chemical dissociation of the sulfonate group, rendering the compound ineffective.[18]

The overall objective of the present study was to evaluate the efficacy and practicality of intracoelomic injection of MS222 for euthanasia in reptiles. The specific objectives were 1) to evaluate whether MS222 can be used to euthanize reptiles and 2) to devise a protocol for preparing concentrated pH-neutralized MS222 that could easily be followed in the laboratory or field for use in euthanasia of reptiles.

Materials and Methods

Animal subjects. Three western fence lizards *(Sceloporus occidentalis*), 2 desert iguanas (*Dipsosaurus dorsalis*), 4 garter snakes (*Thamnophis* spp.), 5 house geckos (*Hemidactylus frenatus*), and 1 flat-tailed house gecko (*H. platyurus*) were housed in an AAALAC-accredited facility and provided with food, housing, lighting, and environmental parameters according to established standard operation procedures for each species. All protocols were approved by the University of California Berkeley Institutional Animal Care and Use Committee.

MS222 preparation. Solutions of 0.7%, 1%, and 1.3% MS222 were prepared by placing 0.7, 1, and 1.3 g MS222 in separate volumetric flasks and adding tap water to total 100 ml each. A 50% (v/v) solution was prepared by adding equal volumes of water and MS222. Sodium bicarbonate then was titrated to each solution until a pH of 7 was reached. The 50% (v/v) solution could not be neutralized as a strong chemical reaction occurred when the buffer was added; a thick oily precipitate remained when the reaction was finished. A heavy precipitate also formed when the 1.3% solution was buffered, and these concentrations were not further evaluated as 'Stage 1' buffered solutions. The 0.7% and 1% solutions were neutralized to pH 7 without heavy precipitate formation, although both solutions were slightly cloudy.

Intracoelomic injection of 0.7% MS222. Two western fence lizards, 2 desert iguanas, and 1 garter snake were weighed and injected intracoelomically with 250 to 500 mg/kg 0.7% MS222 (Table 1). Time to loss of righting reflex and loss of response to painful stimulus (toe or skin pinch) was noted for each animal. A second injection of 50% (v/v; unbuffered) MS222 was given 15 to 25 min after the first injection, when the reptiles began to regain the righting reflex.

Evaluation of 2-stage euthanasia with MS222. Six geckos (weight, 4 to 6 g), 1 western fence lizard (12 g), and 3 garter snakes (8, 8.5, and 300 g) were injected intracoelomically with 250 mg/kg 1% MS222 (Stage 1). As soon as loss to response to toe or skin pinch occurred, the reptiles were injected intracoelomically with 50% (v/v) MS222 as follows (Stage 2): 5 of the 6 geckos were given 0.1 ml, the western fence lizard received 0.3 ml, the 2 smaller garter snakes each received 0.2 ml, and the 300-g snake was given 1.0 ml. One gecko retained a weak response to deep toe pinch 3 min after intracoelomic injection of 1% solution at 250 mg/kg; this animal was injected intracoelomically with 0.2 ml 50% (v/v) MS222 solution at 3 min after the first injection and was observed carefully. The body cavities of all reptiles were opened after 2 to 3 min after cessation of respiration, and hearts were observed for heart beats.

Results

Injection of reptiles with 0.7% MS222 solution at doses of 250 to 500 mg/kg resulted in rapid loss of consciousness in all animals, although response was slower in the snake; a weak response to painful stimulus (a strong skin pinch) remained 12 min after injection of 0.7% solution at 500 mg/kg in this animal (Table 1). Respiratory and cardiac functions remained intact until injection of 50% solution resulted in rapid cessation of breathing and visible heart beat.

In 9 of 10 reptiles, injection of 1% MS222 solution at 250 mg/kg resulted in loss of righting reflex and response to toe pinch within 3 min, but respirations were still present. The remaining animal (a gecko) maintained a weak response to painful stimulus (a strong toe pinch) after 3 min. In all reptiles, respirations and palpable heart beats stopped within 30 to 60 s after injection of the 50% solution. Body cavities were opened 2 min after injection of the 50% solution; hearts had ceased beating in all animals.

No behavioral response (that is, visible evidence of pain) was noted to injection of the 0.7%, 1.0%, or 50% solution in any animal. Postmortem kinking at the injection site did not occur in any animal, including snakes.

Discussion

Euthanasia in reptiles is complex, and no single method is ideal. The method preferred by most users, intracoelomic injection of pentobarbital, is associated with a potential for tissue irritation if highly concentrated formulations are used and a lengthy time to death. In the present study, injection of MS222 resulted in rapid loss of consciousness, followed by cardiac and respiratory arrest, in 15 reptiles. The 2-stage procedure we used fulfills the criteria for euthanasia as outlined in the AVMA guidelines.[1]

Due to the acidity of highly concentrated MS222, euthanasia by this method first requires induction of loss of consciousness by injection of pH-neutralized MS222, followed by euthanasia with the unbuffered 50% (v/v) solution. This protocol is in accordance with the AVMA guidelines and recommendations of other users that allow for a 2-stage euthanasia method, in which the animal first is anesthetized with an injectable agent prior to subsequent administration of euthanasia. Intracoelomic injection of the 50% (v/v) MS222 was performed during Stage 2 of the method described. However, particularly when performing Stage 2 euthanasia in larger MS222-anesthetized reptiles (for example, a 300-g garter snake), handlers comfortable with intracardiac injection in reptiles might choose to perform Stage 2 euthanasia by that route.

Two of the animals (1 garter snake and 1 gecko) retained a weak response to toe or skin pinch after the first MS222 injection. In addition, the tail of 1 snake twitched, and one desert iguana

Table 1. Response of 5 reptiles to intracoelomic injection of 0.7% buffered MS222

Animal	Weight (g)	% MS222	Dose (mg/kg)	Volume given (ml)	Time to loss of righting reflex	Time to no response to toe pinch	Reflexive response	Outcome
Western fence lizard	14	0.7	250	0.5	< 30 s	2 min	open mouth	Occasional gasp. After 20 min, received 0.1 ml 50% MS222. tissue removal after last open mouth response
Western fence lizard	7.5	0.7	500	0.6	< 30 s	3 min	none	After 22 min, limb movement but no response to toe pinch. Tissue removal after 0.1 ml 50% MS222
Desert iguana	44	0.7	500	3.75	< 50 s	2 min	none	After 23 min possible RTP; tissue removal after 0.1 ml 50% MS222
Garter snake	40	0.7	500	3.5	4 min	Still responsive after 12 min. No response to toe pinch 3 min after 0.2 ml 50% MS222	tail twitch	Heart removed 7 min after injection of 50% MS222; no heart beat
Desert iguana	50	0.7	500	4.0	2 min	2 min	none	Tissues removed after injection of 50% MS222

repeatedly opened and closed its mouth. This type of reaction is normal for reptiles. Even foot withdrawals in response to toe pinch have been known to occur after rapid brain destruction and therefore do not indicate consciousness.[11] These quasi-reflexes are attributed to the tolerance of the reptile spinal cord, peripheral nerves, and muscles to hypoxic and hypotensive conditions and to marked integration of somatic responses with the spinal cord instead of the brain.[11] Because of the inability of MS222 to eliminate responses to deep pain stimulus, the use of this drug as an anesthetic for surgery cannot be advocated. However, the level of anesthesia appears to be adequate to eliminate response to the potential irritation due to injection of injected highly concentrated, acidic MS222.

Doubling the dose (500 mg/kg compared with 250 mg/kg) did not shorten the time to onset or improve the quality of anesthesia (Table 1). Fluid was evident in the coelomic cavities, and the limiting factor to the actual dose absorbed is probably the ratio of the surface area to volume injected rather than to the total drug injected. We therefore recommend a dose range of 250 to 500 mg/kg MS222 of 1% solution for the initial injection. A euthanasia protocol that is easily performed in the laboratory or field is provided in Figure 1.

MS222 is the only anesthetic approved by the US Food and Drug Administration for use with food fish.[26] Reversible retinal toxicity occurred in an ichthyologist with a history of long-term skin exposure to MS222,[6] but there are no other published reports of health hazards to handlers. Gloves should always be worn when using MS222, inhalation of the powdered form should be avoided, and eye protection should be worn if there is risk of splashing. MS222 wastewater should be diluted and flushed down the drain into a sanitary sewer system, never to surface water. In field locations, where a sewer is not available, the solution should be further diluted with water and discarded on soil, away from water.

There is no evidence that MS222 has any adverse effects on research. It is considered to lack effects on histopathology[2,27] (although the cited reference[25] does not make this conclusion). An adverse effect of MS222 on African clawed frog *Xenopus* oocytes has been discussed but convincingly argued against.[17] MS222 does not induce primary DNA damage in fish[4] and is considered the anesthetic of choice for anesthesia of transgenic *Xenopus tropicalis*.[13]

In conclusion, MS222 offers an acceptable alternative to sodium pentobarbital for euthanasia of reptiles. Its use is practical in both the laboratory and field and does not require US Drug Enforcement Agency approval. Euthanasia is rapid, and museum specimens can be prepared safely without muscle kinking or mutilation. MS222 therefore meets the criteria for use in reptiles in clinical, research, and museum settings.

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Stepwise procedure for 2-Stage reptile euthanasia with MS222

Step 1: Create 50% (v/v) solution by combining equal volumes of MS222 and water (for example, add 1 teaspoon MS222 into 5 ml water). Mix until all is in solution; the solution should be clear. Label and set aside. Fresh solutions should be made daily prior to use.

Step 2: Create a 1% solution of MS222 using method A or B below. Stir until all is in solution. Each ml of this 1% solution contains approximately 10 mg MS222.

A. Create a **small volume of 1% MS222 solution** (approximately)[a] by adding 0.1 to 0.2 ml 50% (v/v) solution for every 1 teaspoon (5 ml) water.[b] This volume would be appropriate for euthanasia of several small reptiles. A 12-g lizard, for example, requires 0.3 ml 1% solution to receive 250 mg/kg MS222.
B. Create a **large volume of 1% MS222 solution** by adding ¾ level teaspoon of MS222 to 100 ml water. This amount would be appropriate for euthanasia of several large reptiles. A 300-g snake, for instance, requires 7.5 ml 1% solution to receive 250 mg/kg MS222.

Step 3: Titrate the 1% MS222 solution with sodium bicarbonate (baking soda) until the pH reaches 6.5 to 7. Check the pH with litmus paper or a pH meter. The solution will turn slightly cloudy. The amount of sodium bicarbonate needed will depend on water volume and hardness; 100 ml of 1% solution in tap water takes about 1/8 teaspoon sodium bicarbonate.

Step 4: Stage 1 of euthanasia. Inject a volume of 1% solution containing 250 to 500 mg/kg MS222 into the coelom of the reptile.

Step 5: Stage 2 of euthanasia. After loss of righting reflex and response to noxious stimulus (some response to deep toe or skin pinch may remain), inject 0.1 to 1.0 ml (depending on reptile's size) 50% (v/v) MS222 into the reptile's heart or coelom.

Step 6: Perform secondary physical euthanasia (such as pith, decapitate, remove internal organs, freeze) after absence of heart beat is determined.

Step 7: Safely discard all solutions after use.

[a]To more accurately create a 1% solution from a 50% solution, 0.1 ml into 4.9 ml would be required. For practical purposes the 5-ml volume can effectively replace 4.9 ml.
[b]The 50% (v/v) MS222 solution is approximately 35% MS222. Therefore, 0.1 ml of 35% into 5 ml will create a solution approximating 0.7%.

Figure 1. Stepwise procedure for 2-Stage reptile euthanasia by using MS222, including preparation of buffered 1% and unbuffered 50% (v/v) solutions.

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